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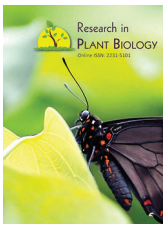

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Screening of antibacterial and phytochemical constituents of *Malva parviflora* Linn. fruit extracts against respiratory tract pathogens

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ABSTRACT

M. parviflora fruit extracts was subjected for extraction with different solvents and tested for antibacterial property by agar well diffusion method against five bacterial pathogens responsible for respiratory tract infections. From this plant, all the studied extracts revealed inhibitory action towards tested microorganisms. Highest inhibition was in methanol and ranged between 15.3±0.28 to 20.6±0.57 mm with uppermost activity against *S. pneumoniae* and lowest against *P. aeruginosa* respectively. The MIC were fixed by two-fold serial dilution method and recorded for methanol extracts at 3.12- 12.5 mg/ml. Phytochemical study revealed the variable presence of secondary metabolites including alkaloids, flavonoids, glycosides, steroids, terpenes, saponins and tannins. From the results, it can be concluded that *M. parviflora* has significant bioactivity against tested microorganisms and can be utilized in drug development against respiratory ailments.

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INTRODUCTION

Numerous microorganisms are commonly associated with aerosols entered into the respiratory system with air and may be a major factor for infections. Microbes can harbour into oropharynx, nasopharynx and nasal cavity, causing sore throat and nasal discharges, in larynx, causing hoarseness, the middle ear, causing otitis media with earache, causing sinusitis with pain in the face or head, and the eyes, causing conjunctivitis or keratitis [1]. Upper respiratory tract is the chief reservoir of opportunistic/commensals bacteria including *Streptococcus pneumoniae*, *Staphylococcus aureus*, *Haemophilus influenzae*, *Moraxella catarrhalis* and *Pseudomonas aeruginosa* [2, 3]. *H. influenzae* and *M. catarrhalis* are also responsible equally for Community Acquired Pneumonia (CAP) in lower respiratory tract. Some atypical pathogens i.e. *Chlamydia pneumoniae*, *Mycoplasma pneumoniae* and *Legionella pneumophila* are apparently present in CAP [4]. Even respiratory viruses including adenovirus, respiratory syncytial virus, influenza virus, human coronavirus and human rhinovirus are recently reported in clinical observation under respiratory infections [5].

Bacteria are capable of causing serious diseases becoming resistant to common antibiotics. The presence of phytochemicals is found in plants those played a major role in disease control as well as in enhancement of human health also. Investigations suggest that phenols, polyphenols, quinine, flavanones, flavonoids, tannins, coumarins, terpenoids, glycosides and saponins are few examples of such phytochemicals which act as antimicrobial, antidiarrhoeal, anthemintic etc. [6-10].

Malva genus is widespread in tropical and temperate region belongs to the family Malvaceae. They possess efficient role in coughing, intestinal infections, colitis, tonsillitis, gastroenteritis, cholesterol and lipid-lowering, antihypertensive, antioxidant, analgestics, emollient, pectoral girdle and arteriosclerosis treatment [11]. *Malva parviflora* Linn. is widely distributed in Uttarakhand. It is originated from the Mediterranean region of Europe and temperate Asia. Halvorson and Guertin [12] has been reported its medicinal uses and as food in many cultures since 6000 B.C. It prefers to grow in shade or slightly sunny area. It is an annual herb, commonly known as cheeseweed [13], marshmallow or little mallow with 40 to 60 cm in height. Flowers are white in colour. Plants of this family are distinguished for their antibacterial and antifungal activities due to the incidence of alkaloids, essential oils and phenolic quleoside [14, 15]. It contains wide range of cytokines which are valuable in treatment of inflammation [16] and aerial parts with high concentration of proteins [17]. *M. parviflora* root

extracts is used for the treatment of dysentery [18], leaves and seeds extracts for teeth infections, as laxative, expectorant and antitussive and in combination with *Eucalyptus* leaves, it helpful in coughing and other chest ailments [19, 20].

The literature survey showed very limited reports describing the antibacterial role of *M. parviflora*. Therefore, herein we demonstrate, antibacterial screening of crude extracts of *M. parviflora* were tested against selected respiratory tract pathogens.

MATERIALS AND METHODS

Plant Material

Fruits of *M. parviflora* were collected from Divya nursery, Patanjali Yogpeeth, Haridwar. Plant was identified at Botanical Survey of India (BSI), Dehradun and deposited a herbarium voucher specimen (BSD 113406). Fruits were cleaned in running water, dried out at room temperature and crushed in an electric grinder.

Preparation of Extracts

The fruit extracts were prepared with petroleum ether (PET), acetone (ACE), methanol (MeOH) and aqueous (H_2O) by following standard method [21] and as explained previously [33].

Microorganisms Used

The bacterial strains namely *Haemophilus influenzae* (MTCC 3826), *Pseudomonas aeruginosa* (MTCC 2474), *Staphylococcus aureus* (MTCC 1144), *Streptococcus pyogenes* (MTCC 422) and *Streptococcus pneumoniae* (MTCC 655) were purchased from Institute of Microbial Technology (IMTECH), Chandigarh and maintained at 4°C on nutrient agar medium. For experiments, active bacterial cultures were prepared by transferring a loopful of cells from stock cultures to test tubes of Mueller Hinton Broth (MHB) and incubated without shakeup for 24 h at 37°C.

Antibacterial Testing

The antibacterial activity was determined by standard method [21].

Determination of Minimum Inhibitory Concentrations (MICs)

Two-fold serial dilution method [22] was employed to evaluate the minimum inhibitory concentration (MIC).

Phytochemical Screening

The phytochemical analysis of plant extracts was done by following the method of Evans [23] and Scalbert [24].

RESULTS AND DISCUSSION

M. parviflora showed moderate antibacterial properties against selected microorganisms (Table 1). The MeOH extract of *M. parviflora* was found most active against all selected bacterial pathogens followed by H_2O, ACE and PET. The maximum inhibition was found against *S. pneumoniae* (20.6±0.57 mm) and lowest against *P. aeruginosa* (15.3±0.28 mm) respectively. The moderate antibacterial activity was noted against *S. aureus* (16.6±0.57 mm), *S. pyogenes* (16.6±0.28 mm) and *H. influenzae* (16.0±0.50 mm). These findings can be justified with its medicinal uses reported by other researchers. According to Islam *et al.* [25] (2007-2010) hexane, chloroform ($CHCl_3$) and ethanol (EtOH) extracts of *M. parviflora* displayed activity against four bacteria i.e. *B. subtilis*, *E. coli*, *S. aureus* and *P. vulgaris*. EtOH extract of leaves and seeds showed activity against *B. Subtilis*, *K. pneumoniae*, *S. aureus*, *E. aerogenes* and *P. aeruginosa* [26]. The ethanol extract of *M. parviflora* leaves had been reported a significant neuroprotective effect as by decreasing in escape latency (EL) and significant increase in time spent in the target quadrant (TSTQ), kind of improvement of learning and memory in mice [27].

In another study, three antimicrobial proteins i.e. CW-3, CW-4 and CW-5 were purified from seeds of *M. parviflora*. CW-3 and CW-4 proteins showed activity against *Phytopthora infestans* [28]. Some study supports *M. parviflora* as a good source for synthesis of nanomedicine [29].

The sensitivity of tested bacterial strains was quite interesting in respect to reference drug (erythromycin). In comparison to erythromycin, the activity of *M. parviflora* fruit extracts was found less efficient. Erythromycin is a macrolide antibiotic with broad spectrum antimicrobial role. In case of respiratory tract infections, it has better treatment of microorganisms especially for atypical organisms i.e. Mycoplasma and legionellosis [30].

The MICs were calculated for MeOH extract (Figure 1) and found to be varied at 3.12 - 12.5 mg/ml. The minimum inhibition was observed against *S. pneumoniae* at 3.12 mg/ml and maximum against *P. aeruginosa* at 12.5 mg/ml respectively. *H. influenzae*, *S. aureus* and *S. pyogenes* showed similar MICs at 6.25 mg/ml.

The preliminary phytochemical analysis for *M. parviflora* fruit extracts showed the presence of alkaloids, flavonoids, glycosides, steroids, terpenes, saponins and tannins in MeOH extract, alkaloids and flavonoids in PET extract, alkaloids, flavonoids, steroids, terpenes and saponins in ACE extract, and flavonoids, steroids, terpenes, saponins and tannins in H_2O extract (Table 2). The results can be justified with other co-workers finding which concluded the presence of major secondary metabolites including flavonoids, alkaloids, glucosides, tannins, saponins in various parts of *M. parviflora*.

Farhan *et al.* [31] had showed the presence of flavonoids, tannins, phenols, saponins, alkaloids and resin in stem and leaves of *M. parviflora*. Abdel-Ghani *et al.* [11] had reported seven compounds including β- amyrin, α-amyrin, a mixture of β sitosterol and stigmasterol, cholesterol, campasterol, ergosterol, and β- sitosterol-O-β-D-glucoside in petroleum fraction, ethyl vanillin in chloroform fraction and tribuloside, a flavonoid glycoside, in ethyl acetate fraction. If we focus on the

Table 1: Antibacterial activity of *Malva parviflora*

Microorganisms	*Diameters of inhibition zone (mm)				DMSO	Positive Control (Erythromycin)
	PET	ACE	MeOH	H2O		
H. influenzae	8.3±0.28	12.3±0.28	16.0±0.50	11.3±0.28	-	21.3±0.76
P. aeruginosa	9.3±0.28	11.3±0.28	15.3±0.28	12.3±0.07	-	16.6±0.76
S. aureus	9.0±0.50	11.6±0.28	16.6±0.57	13.2±0.12	-	30.3±0.76
S. pneumoniae	9.6±0.28	14.0±0.50	20.6±0.57	15.8±0.10	-	19.3±0.57
S. pyogenes	9.0±0.50	13.3±0.76	16.6±0.28	13.4±0.10	-	25.6±0.28

*Values are mean±SE, - : No inhibition, Cork borer diameter: 6 mm, PET: Petroleum ether, ACE: Acetone, MeOH: Methanol, H2O: aqueous, DMOS: Dimethyl sulfoxide

Table 2: Screening of phytochemical from *M. parviflora*

S. No.	Phytoconstituents	Solvents			
		PET	ACE	MeOH	H2O
1.	Alkaloids	+	+	+	-
2.	Flavonoids	+	+	+	+
3.	Glycosides	-	-	+	-
4.	Steroids	-	+	+	+
5.	Terpenes	-	+	+	+
6.	Saponins	-	+	+	+
7.	Tannins	-	-	+	+

+ : Present, - : Absent, PET: Petroleum ether, ACE: Acetone, MeOH: Methanol, H2O: aqueous

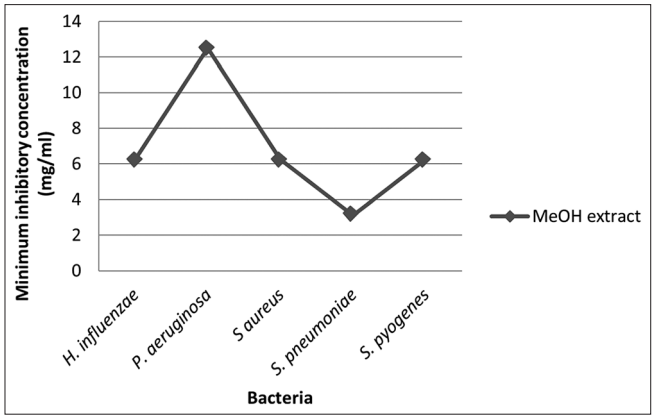

Figure 1: Minimum Inhibitory Concentrations (MICs) of MeOH extract of *M. parviflora*. The inhibition is noted at (a) 6.25 mg/ml against *H. influenzae*, *S. aureus* and *S. pyogenes* (b) 12.5 mg/ml against *P. aeruginosa* (c) 3.12 mg/ml against *S. pneumoniae*

biological properties of these phytoconstituents, a wide range of activity can be observed. Phytochemicals are responsible for colour, odour etc. and bearing protective or disease preventive properties. They are accountable for some other properties including antioxidant activity, hormonal action, enzymatic activity, interference with DNA replication, antimicrobial activity etc. [32].

CONCLUSION

In conclusion, *M. parviflora* fruit extracts have moderate antibacterial properties against selected microorganisms. The bioactivity of fruit extracts was confirmed by the results of phytochemical analysis. This can be utilized in respiratory tract illnesses caused by studied microorganisms. Further, research should be carried out to explore the bioactive components of *M. parviflora*.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interests.

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REFERENCES

1. Collee JG, Duguid JP, Fraser AG, Marmion BP, Simmons A. Laboratory strategy in the diagnosis of infective syndromes. In: Mackie & McCartney Practical Medical Microbiology, Collee JG, Marmion BP, Fraser AG, Simmons A, editors. 1996; pp 56-64. ISBN:044-304-9068
2. Brook I. Microbiology of sinusitis. Proc. Am. Thorac. Soc. 2011;8(1):90-100. doi:10.1513/pats.201006-038RN
3. Bosch AATM, Biesbroek G, Trzcinski K, Sanders EAM, Bogaert D. Viral and bacterial interactions in the upper respiratory tract. PLoS Pathog. 2013;9(1):e1003057. doi:10.1371/journal.ppat.1003057
4. Guthrie R. Community acquired lower respiratory tract infections: etiology and treatment. Chest 2001;120(6):2021-2034. doi:10.1378/chest.120.6.2021
5. Ryu S, Kim S, Kim BI, Klein EY, Yoon YK, Chun BC. Temporal relationship between antibiotic use and respiratory virus activities in the republic of Korea: A time-series analysis. Antimicrobial Resistance & Infection Control. 2018;7:56. doi: 10.1186/s13756-018-0347-8
6. Cowan MM. Plant products as antimicrobial agents. Clinical Microbiology Review. 1999;12(4):564-582.
7. Mali RG, Mahajan SG, Mehra AA. *In vitro* anthelmintic activity of stem bark of *Mimusops elengi* Linn. Pharmacognosy magazine. 2007;3(10):73-76.
8. Wang GS, Han J, Zhao LW, Jiang DX, Liu YT, Liu XL. Anthelmintic activity of steroidal saponins from *Paris polyphylla*. Phytomedicine 2010;17:1102-1105.
9. Shaibani TRMA, Phulan MS, Shiekh M. Anthemintic activity of *Fumaria parviflora* (Fumariaceae) against gastrointestinal nematodes of sheep. Int. J. Agric. Biol. 2009;11:431-436.
10. Bachaya HA, Iqbal Z, Khan MN, Jabbar A, Gilani AH, Din IU. *In vitro* and *in vivo* anthelmintic activity of *Terminalia arjuna* bark. Int. J. Agric. Biol. 2009;11:273-278.
11. Abdel-Ghani AE, Hassan HM, El-Shazly AM. Phytochemical and biological study of *Malva parviflora* L. Grown in Egypt. Zagazig. J. Pharm. Sci. 2013;22(1):17-25.
12. Halvorson WL, Guertin P. Factsheet for: *Malva parviflora* L. USGS weeds in the west project: Status of introduced plants in Southern Arizona Parks, 2003.
13. Wang X, Bunkers GJ. Potent heterologous antifungal proteins from cheeseweed (*Malva parviflora*). Biochem. Biophys. Res. Commun.

2000;279(2):669-673. doi:10.1006/bbrc.2000.3997
14. Ndunga M, Oumba JM. Antimicribial and antifungal activities of essential oils of *Ocimum grattisimum* and *Ocimum basilicum* from Congo. Fitotherapia 1997;97(2):190-191.
15. Abad MJ, Ansuategui M, Bermejo P. Active antifungal substances from natural sources. Arkivoc. 2007;2:116-145.
16. Shale TL, Stirk WA, Staden JV. Variation in antibacterial and anti-inflammatory activity of different growth forms of *Malva parviflora* and evidence for synergism of the anti-inflammatory compounds. J. Ethnopharmacol. 2005;96(1-2):325-330. doi:10.1016/j.jep.2004.09.032
17. Ereifej KI, Feng H, Rababah T, Almajwal A, Datt MA, Gammoh SI, Oweis LI. Chemical composition, phenolics, anthocyanins concentration and antioxidants activity of ten wild edible plants. Food and Nutrition Sciences. 2015;6:581-590. doi:10.4236/fns.2015.67061
18. Hernandez T, Canales M, Avila JG, Duran A, Caballero J, Vivar ARD, Lira R. Ethnobotany and antibacterial activity of some plants used in traditional medicine of Zapotitlan de las Salinas, Puebla (Mexico). J. Ethnopharmacol. 2003;88:181-188. doi:10.1016/S0378-8741(03)00213-7
19. Nimri LF, Meqdam MM, Alkofahi A. Antibacterial activity of Jordanian medicinal plants. *Pharm. Biol.* 1999;37:196-201.
20. Akbar S, Hanif U, Ali J, Ishtiaq S. Pharmacognostic studies of stem, roots and leaves of *Malva parviflora* L. Asian Pacific Journal of Tropical Biomedicine. 2014;4(5):410-415. doi:10.12980/APJTB.4.2014C1107
21. Ahmad I, Mehmood Z, Mohammad F. Screening of some Indian medicinal plants for their antimicrobial properties. J. Ethnopharmacol. 1998;62:183-193.
22. Aboaba OO, Smith SI, Olude FO. Antibacterial effect of edible plant extract on *Escherichia coli* 0157:H7. Pakistan Journal of Nutrition. 2006;5(4):325-327.
23. Evans WC. Trease and Evans Pharmacognosy, 5th ed. India: Harcourt Brace and Company; 1996.
24. Scalbert A. Antimicrobial properties of tannins. Phytochemistry 1991;30:3875-3883.
25. Islam M, Ali E, Saeed MA, Jamshaid M, Khan MTJ (2007-2010). Antimicrobial and irritant activities of the extracts of *Malva parviflora* L., *Malvastrum coromandelianum* L., and *Amaranthus viridis* L.- a preliminary investigation. Pakistan Journal of Pharmacy. 2007-2010;20-23(1&2):3-6.
26. Miri A, Rad JS, Alfatemi SMH, Rad MS. A study of antibacterial potentiality of some extracts against multidrug resistant human pathogens. Annals of Biological Research 2013;4(8):35-41.
27. Aslam M, Sial AA. Neuroprotective effect of ethanol extract of leaves of *Malva parviflora* against Amyloid-β-(Aβ) mediated Alzheimer's disease. International Scholarly Research Notices. 2014; doi: 10.1155/2014/156976
28. Wang X, Bunkers GJ, Walters MR, Thoma RS. Purification and characterization of three antifungal proteins from cheeseweed (*Malva parviflora*). Biochem. Biophys. Res. Commun. 2001;282(5):1224-1228. doi:10.1006/bbrc.2001.4716
29. Zayed MF, Eisa WH, Shabaka AA. *Malva parviflora* extract assisted green synthesis of silver nanoparticles. Spectrochim. Acta Mol. Biomol. Spectrosc. Spectrochim. Acta. Mol. Biomol. Spectrosc. 2012;98:423-428. doi:10.1016/j.saa.2012.08.072
30. Kwon JH. Macrolides, ketolides, lincosamides and streptogramins. In: Cohen J, Powderly WG, Opal SM, editors. Infectious Disease, 4th ed. 2017;pp 1217-1229.e1, doi: 10.1016/B978-0-7020-6285-8.00141-6
31. Farhan H, Rammal H, Hijazi A, Hamad H, Daher A, Reda M, Badran B. In vitro antioxidant activity of ethanolic and aqueous extracts from crude *Malva parviflora* L. Grown in Leanon. Asian J. Pharm. Clin. Res. 2012;5(3):234-238.
32. Doughari JH. Phytochemicals: Extraction methods, basic structures and mode of action as potential chemotherapeutic agents. In: Phytochemicals- A Global Perspective of their Role in Nutrition and Health, Venketeshwer Rao editor. 2012; p. 1-33. ISBN: 978-953-51-0296-0
33. Gautam SS, Navneet Kumar S. Appraisal of antimicrobial properties of *Onosma bracteatum* Wall. fruit extracts against respiratory tract pathogens. Journal of Medicinal Herbs and Ethnomedicine. 2015;1:108-112.